

## In Vitro Antifungal Susceptibilities of *Trichosporon* Species

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**The in vitro activities of amphotericin B, itraconazole, fluconazole, voriconazole, posaconazole, and ravuconazole against 39 isolates of *Trichosporon* spp. were determined by the NCCLS M27-A microdilution method. The azoles tested appeared to be more potent than amphotericin B. Low minimal fungicidal concentration/MIC ratios were observed for voriconazole, posaconazole, and ravuconazole, suggesting fungicidal activity.**

*Trichosporon* spp. are well recognized as pathogens capable of causing invasive disease (1, 2, 8). Trichosporonosis, the deep-seated infection caused by this fungus, is an often fatal disease affecting mostly immunocompromised hosts (1, 3, 4, 8, 10, 12). Mortality is as high as 80% (8, 9, 12, 19), and it has been reported as the most common cause of noncandidal yeast infection in patients with hematological malignancies (9). Since the taxonomic revision of the genus *Trichosporon* in 1992 (6), it has been recognized that the formerly used designation *Trichosporon beigeli* includes several genetically distinct species. *T. asahii* is the species most often implicated in deep-seated infections in humans.

Despite the increased frequency and severity of trichosporonosis, data on the antifungal susceptibilities of *Trichosporon* spp. are limited (5, 7, 11, 15–18) and recommendations for in vitro testing of this fungus are not included in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Treatment of this infection with the currently available antifungal agents has generally been unrewarding, and clinical failures of amphotericin B (AMB), fluconazole (FLU), and a combination of the two have been reported (1, 3, 8, 12, 13, 20). Investigational triazoles such as voriconazole (VOR), posaconazole (POS), and ravuconazole (RAV) are attractive candidates for the treatment of this infection. With this study, we sought to determine the in vitro activities of various antifungals against *Trichosporon* spp., as well as to gain experience with the NCCLS M27-A method application for this organism, utilizing the largest number of isolates tested so far in one setting.

A total of 39 *Trichosporon* sp. clinical isolates were evaluated. These consisted of 24 strains of *T. asahii*, 10 of *T. mucoides*, and 5 of *T. inkin*, which were identified twice by API 20C AUX system (BioMérieux Vitek Inc., Hazelwood, Mo.) testing. The isolates were maintained in water stocks and subcultured twice on Sabouraud dextrose agar (SDA) plates with incubation at 35°C for 24 to 48 h. Four quality control *Candida* strains (*Candida krusei* ATCC 6258, *C. parapsilosis* ATCC 22019, *C. lusitaniae* 5W31, and *C. lusitaniae* CL524) were also

included in the experiments. VOR and FLU (Pfizer Central Research, Sandwich, United Kingdom), RAV and AMB (Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, N.J.), ITRA (Janssen Pharmaceutica, Beerse, Belgium), and POS (Schering-Plough Research Institute, Bloomfield, N.J.) were obtained from the manufacturers as defined powders. The NCCLS M27-A microdilution broth-based method (14) was used to determine MICs. Drug stocks and dilutions were prepared in dimethyl sulfoxide (Sigma Chemical Co., St. Louis, Mo.), except for FLU, which was prepared in sterile distilled water. Final drug concentrations in the microdilution plates ranged from 64 to 0.125 µg/ml for FLU and from 16 to 0.031 µg/ml for all of the other drugs. The microdilution plates were prepared by using the synthetic medium RPMI 1640 (Sigma Chemical Co.) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Sigma Chemical Co.). In addition, AMB was also tested in Antibiotic Medium 3 buffered to pH 7.0 by addition of 0.01 M phosphate (AM3; Becton Dickinson and Co., Cockeysville, Md.). Yeast suspensions were spectrophotometrically prepared after vortexing and adjustment to a 0.5 McFarland standard transmittance at a wavelength of 530 nm. After serial dilution of the above-described suspensions, the final inoculum yielded was  $0.5 \times 10^3$  to  $2.5 \times 10^3$  cells/ml, which was confirmed by performing colony counts on SDA plates. MICs were visually determined at 24 and 48 h of incubation at 35°C. As accurate spectrophotometric MIC determinations were not possible due to the speckled growth pattern that the organisms exhibited in the microdilution plates, the visually determined MIC was defined as the lowest drug concentration at which there was complete absence of growth (MIC-0) or a prominent growth reduction (MIC-2) in comparison with that of controls. Minimum fungicidal concentration (MFC) determinations were done for VOR, POS, and RAV by plating 20 µl of each clear well on SDA plates after 48 h of incubation at 35°C. The MFC was the lowest drug concentration giving no growth on the SDA plates ( $\geq 98\%$  killing). Experiments were repeated in duplicate, and when discordant MICs were found, the higher one was reported. As a means by which to estimate concordance between MICs and MFCs, MIC/MFC ratios were calculated for each strain and the range and median of these ratios are reported (Table 1).

AMB, FLU, and ITRA MICs against the American Type Culture Collection (ATCC) quality control *Candida* strains

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TABLE 1. MICs and MFCs of various antifungal agents against 39 clinical *Trichosporon* sp. isolates<sup>a</sup>

<i>Trichosporon</i> sp. (no of isolates) and antifungal agent/medium	MIC <sub>50</sub> (range)		MFC <sub>50</sub> (range)	Median MFC/MIC-0 ratio (range)
	MIC-2	MIC-0		
<i>T. asahii</i> (24)				
AMB/AM3	ND	4 (1–8)	ND	ND
AMB/RPMI	ND	0.5 (0.25–8)	ND	ND
FLU/RPMI	2 (0.5–>64)	8 (4–>64)	ND	ND
ITRA/RPMI	0.12 (0.06–>16)	0.25 (0.12–>16)	ND	ND
RAV/RPMI	0.5 (0.25–>16)	2 (1–>16)	4 (1–>16)	1.5 (1–16)
POS/RPMI	0.12 (0.06–>16)	0.5 (0.25–>16)	1 (0.5–>16)	1 (1–8)
VOR/RPMI	0.06 (0.03–>16)	0.25 (0.06–>16)	0.5 (0.06–>16)	1 (1–>16)
Non- <i>T. asahii</i> (15)				
AMB/AM3	ND	1 (0.25–8)	ND	ND
AMB/RPMI	ND	0.25 (0.06–1)	ND	ND
FLU/RPMI	2 (0.5–4)	8 (1–>64)	ND	ND
ITRA/RPMI	0.12 (0.03–0.5)	0.25 (0.12–>16)	ND	ND
RAV/RPMI	0.5 (0.03–>16)	4 (0.25–>16)	>16 (0.25–>16)	1 (1–16)
POS/RPMI	0.12 (0.03–0.5)	1 (0.25–>16)	8 (0.25–>16)	1 (1–16)
VOR/RPMI	0.06 (0.03–0.25)	0.25 (0.06–>16)	16 (0.06–>16)	1 (1–>16)

<sup>a</sup> ND, not done. Data are shown for 48 h and expressed in micrograms per milliliter, except for the MIC/MFC ratio.

were within the acceptable limits determined by the NCCLS. Fungal growth was readily visible by 24 h of incubation and somewhat increased by 48 h. Results are shown in Table 1. The MIC-0 was the endpoint used for AMB MIC determination, whereas both the MIC-0 and the MIC-2 are reported for the azoles. MICs at 24 and 48 h were within 1 or 2 dilutions of each other. AMB MICs were relatively high for both *T. asahii* and non-*T. asahii* isolates and were eightfold higher when AM 3 was the medium used for AMB testing. High AMB MFC/MIC ratios were previously reported by Walsh et al. and correlated with poor clinical outcomes in neutropenic patients (20). Other investigators have also reported relatively high AMB MICs for limited numbers of isolates tested (7, 16–18). Our results confirm these previous observations, suggesting that AMB treatment alone for trichosporonosis is unlikely to be rewarding, especially when host defenses are lacking.

FLU MICs were relatively low, confirming previous findings of in vitro activity of FLU against *Trichosporon* sp. isolates (7, 15, 16, 18). Unfortunately the in vitro observation of FLU activity has not correlated well with good clinical outcomes. ITRA MICs were similarly relatively low. Our results confirm the previous observation of Perparim et al. (16), as well as of other authors (7, 11, 15, 17, 18), that the azoles, in general, appear to be more active in vitro against this pathogen compared to AMB. However, high MFCs of both FLU (MFC<sub>50</sub> [MFC for 50% of the strains tested], >80 µg/ml) and ITRA (MFC<sub>50</sub>, >10 µg/ml) have been previously reported (20). Animal data have also shown superiority of FLU, compared to AMB, in prolonging survival time and reducing the fungal burden in the kidneys of neutropenic mice (21).

RAV, POS, and VOR appeared to be more active than AMB and FLU and similar to ITRA. The MIC-0 and MIC-2 of the investigational triazoles were often similar, but some isolates did show significant trailing. The MIC-0 and MFC correlated closely for some isolates, suggesting fungicidal activity, but the MICs and MFCs for other isolates were high. Based on the MIC-0 and MFC, the non-*T. asahii* isolates appeared more resistant to the investigational triazoles than did the *T. asahii* isolates but more susceptible to AMB. VOR and POS ap-

peared to be slightly more potent in vitro than RAV. Although there are no established breakpoints to define antifungal susceptibility for *Trichosporon* spp., our study suggests a superiority of the azoles to AMB and a potential fungicidal effect of POS, VOR, and RAV. Further studies for in vivo correlation of these findings seem worthwhile.

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